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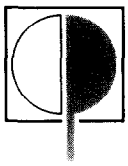

# Effects of herbicides on *in vitro* vegetative growth and sporulation of entomopathogenic fungi

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Four commonly used herbicides were evaluated at two different temperatures and three concentrations (10X, 1X and 0.1X, where X = recommended field rate) for compatibility *in vitro* with the entomopathogenic fungi *Conidiobolus thromboides*, *C. coronatus*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces farinosus* and *Verticillium lecanii*. The effects of the herbicides on radial growth during the whole test period (14 days at 25°C and 24 days at 15°C), and intensity of sporulation were quantified. Generally, phenmedipham plus desmedipham (1:1), metolachlor, and chloridazon had pronounced adverse effects on all fungi. The 1X rate of these three herbicides either totally impaired the development of the fungi at both temperatures or strongly inhibited the growth at 25°C, as in *B. bassiana*, *M. anisopliae* and *V. lecanii*. The 0.1X rate of these herbicides usually resulted in fungistasis, which was not expressed in subcultures of the fungi on herbicide-free agar. The fourth herbicide, lenacil, killed *C. thromboides* when tested at 25°C at the 1X and 10X rates. At the 0.1X rate, lenacil had a temporary stimulatory effect on the development of the test fungi. In all other fungus–herbicide concentration–temperature combinations, lenacil had a temporary, reversible inhibitory effect. Of the herbicides tested, lenacil, which showed a less adverse effect in the tests, is probably compatible with most entomopathogenic fungi in the field. This is not the case, however, with phenmedipham plus desmedipham, metolachlor, and chloridazon, which are strongly antagonistic.

**Keywords:** Herbicide compatibility; entomopathogenic fungi; vegetative growth

Regular application of a wide spectrum of agrichemicals including pesticides can result in their accumulation in the environment and in a change in its biological component through alteration of certain links in the chain of biocenotic interrelations. An emphasis on the role of natural enemies is one of the key approaches in integrated pest management. Most natural enemies, on which biological control of pest species depends, have been found to be sensitive to chemical pesticides (Havron, Rosen and Rössler, 1987). Lack of pesticide resistance/tolerance in biological control agents is therefore a serious hindrance to increasing their role and efficacy in integrated pest management programmes. Selective pesticides that can be used to control pests without adversely affecting important natural enemies are urgently needed.

Several entomopathogenic fungi play a significant role in the natural control of pest arthropods (Keller, 1986; Tanada and Kaya, 1993). Pesticides are anthropogenic environmental factors that affect insects and their pathogens. Because pesticides may synergize or antagonize disease in insects, they may be regarded as epizootiologically relevant factors (Benz, 1987).

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Herbicides are widely and routinely used crop protection chemicals (Cernáková, Kurucová and Fuchsová, 1991) and may exert positive or negative influences on non-target organisms such as soil fungi and bacteria (Wilkinson and Lucas, 1969; Balicka, 1970; Wojciechowska, Bajan and Kmitowa, 1977; Wardle and Parkinson, 1992). The soil is where part of the life cycle of many entomopathogenic fungi takes place. Studies have indicated that proper selection and scheduling of pesticides is necessary to minimize their deleterious effects on entomopathogenic fungi (Storey and McCoy, 1992).

The complexity and cost of large-scale and long-dated field studies underscore the need for initial toxicity tests in the laboratory, although it is clear that extrapolation of results *in vitro* to practices in the field is difficult and conjectural (Moorhouse *et al.*, 1992). Nevertheless, experiments *in vitro* allow selected products to be further tested *in vivo* in the laboratory (and ultimately in the field) and allow justifying that selection. Our laboratory investigation was conducted to determine the effects of commonly used herbicides on the growth and sporulation of selected entomopathogenic fungi of the Entomophthorales and Hyphomycetes. We present data showing that several

herbicides tested are injurious to certain fungi and comment on the implications of our findings.

## Materials and methods

Details of the test herbicides are listed in *Table 1*.

Chloridazon is a pre-emergence and early post-emergence herbicide for use in sugar, red and fodder beet crops. Lenacil is used primarily in Europe for weed control in sugar beets, cereal grains and strawberries. Metolachlor is marketed for pre-emergence and preplant-incorporated weed control in corn, soybeans, peanuts, grain sorghum, potatoes, pod crops, safflower, and woody ornamentals; it is also selective on sunflowers, flax and certain other crops. Phenmedipham plus desmedipham (1:1) is a post-emergence herbicide for control of such weeds as lambsquarters (*Chenopodium album* L), *Brassica* species, and green foxtail [*Setaria viridis* (L.) P. Beauv.] in red table and sugar beets, spinach and strawberries.

The herbicides were tested for compatibility with entomopathogenic fungi at the commercial concentration of active ingredient [based on the manufacturers' recommended rates for field use (1X), *Table 1*] per litre of culture medium and at 0.1 times (0.1X) and 10 times (10X) the recommended rate. Distilled acetone was used as the solvent.

Aliquots (5 ml) of each acetone solution of herbicide were added to 1 l autoclaved Sabouraud dextrose agar (SDA) cooled to 45°C. The ingredients were thoroughly mixed and 20 ml of the mixture were poured into 10 cm glass Petri dishes. SDA treated only with 5 ml acetone $l^{-1}$ was used as control. A small plug of SDA (1 mm deep × 2 mm in diameter) was removed from the centre of each dish.

Inocula of the test fungi were produced on slants of SDA. Slants were incubated for 2–3 weeks at 25 ± 1°C and spores were harvested from the surface cultures by scraping and suspending in sterile distilled water. The spore viability (formation of germ tubes) of each fungus was assessed on 1.5% water agar and quantified using a Fuchs–Rosenthal cell-counting chamber (with the sturdy, small spores of Hyphomycetes) or a serial dilution technique (with the fragile, large spores of Entomophthorales). Germination rates were over 95% for all test fungi. One 0.1 ml aliquot of an aqueous suspension containing 500 ± 50 viable fungal spores was pipetted into the well in each dish, which then was sealed with parafilm. The fungi used in the study, retrieved from the culture collection of the Akademia Rolnicza, Szczecin, Poland, included the entomophthoraleans *Conidiobolus coronatus* (Costantin) Batko and *C. thromboides* Drechsler, and the hyphomycetes *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Paecilomyces farinosus* (Holm ex SF Gray) Brown & Smith and *Verticillium lecanii* (Zimmerman) Viégas. Each herbicide–concentration–fungus combination and corresponding control was replicated eight times.

Four replicates of each combination and control were incubated at 15°C and four at 25°C, in complete darkness. It is agreed that the optimal developmental temperature of the entomopathogenic fungi is generally 15–25°C for the Entomophthorales and 20–30°C for the Hyphomycetes (Roberts and Campbell, 1977). Radial mycelial growth was measured from the edge of the well on days 3, 5, 7, 9, 12 and 14 of incubation at both temperatures, and again on day 24 at 15°C. All measurements were taken with a millimetric ruler along the same premarked radial line. The replicated values recorded at each reading were averaged (±s.e.) and the growth of each fungus on herbicide-treated SDA was expressed as a percentage of growth of the corresponding control.

Sporulation intensity, if any, and spore viability were determined, as described earlier, on day 14 (at 25°C) and 24 (at 15°C) after inoculation. Data were expressed as percentage of reduction or enhancement in production of viable spores $cm^{-2}$ of fungal colony on herbicide-treated SDA in comparison to controls (Hokkanen and Kotiluoto, 1992). The herbicides were further classified in evaluation categories (harmless, slightly harmful, moderately harmful, and harmful in initial toxicity tests *in vitro*) according to Hassan's classification scheme (Hassan, 1989).

To distinguish between a fungistatic/antibiotic (inhibition of germination of fungal spores/of vegetative growth) and a fungicidal (kill of spores) effect, the original inoculum (spores or spores plus hyphae) was transferred from herbicide-treated SDA to fresh, untreated SDA 14 (at 25°C) and 24 (at 15°C) days after inoculation. One $cm^2$ plug of SDA containing the original inoculation well was removed with a cutting device and placed in 10 ml sterile water. After 5 min agitation on a magnetic stirrer, 2 ml of the aqueous fungus suspensions were evenly distributed on the

Table 1. Details of the test herbicides

| Common name (Trade name) | Recommended rate (% a.i.) | Chemical name (IUPAC) | Manufacturer |
|---|---|---|---|
| Chloridazon (Pyramin WP) | 1.0 | 5-Amino-4-chloro-2-phenyl pyridazin-3-(2*H*)-one | Z. Ch. Organika-Azot, Poland |
| Lenacil (Venzar WP) | 0.25 | 3-Cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2, 4(3*H*)-dione | Z. Ch. Organika-Zarów, Poland |
| Metolachlor (Dual 720 EC) | 0.5 | 2-Chloro-6'-ethyl-*N*-(2-methoxy-1-methylethyl) acet-*o*-toluidide | Ciba-Geigy Ltd, Switzerland |
| Phenmedipham + desmedipham (1:1) (Betanal EC) | 2.0 | Methyl 3-(3-methylcarbaniloyloxy)carbanilate + ethyl 3'-phenylcarbamoyloxycarbanilate (1:1) | Z. Ch. Organika-Azot |

surface of untreated SDA in 10 cm Petri dishes. The dishes were incubated for 14 days at 25°C, in complete darkness. The effect of the herbicide was scored as fungicidal if no development occurred on SDA and as fungistatic/antibiotic otherwise.

## Results

For conciseness, the effects of the herbicides on growth of the fungi and intensity of production of viable spores are summarized *only* for day 14 (at 25°C) and day 24 (at 15°C) after inoculation. Data from other times of measurement of radial growth do not differ from those reported for days 14 and 24. When warranted, data from other times are reported in the text.

### Phenmedipham plus desmedipham and metolachlor

Phenmedipham plus desmedipham (1:1) (carbamates) and metolachlor (an acetamide) were fungicidal to *C. coronatus* and *C. thromboides* at the two temperatures and three rates of active ingredient tested. Vegetative growth and sporulation were not observed (*Tables 2–4*). Spores from the original inoculum transferred from herbicide-treated to untreated agar did not germinate.

Fungicidal activity also was observed against the four Hyphomycetes at both temperatures at the 10X rate (*Table 4*), and at 15°C at the 1X rate (*Table 2*). Spores did not germinate after transfer to untreated agar. At 25°C, the herbicides tested at the 1X rate were fungicidal to *P. farinosus* (no germination of spores transferred to untreated agar) and fungistatic to the three other Hyphomycetes until between days 7 and 9 of incubation (data not shown). The fungistasis was partially overcome thereafter, although the radial growth on day 14 of *B. bassiana*, *M. anisopliae* and *V. lecanii* was <20% compared with controls and production of viable spores was markedly reduced (*Table 2*). Transfers to herbicide-free agar of the latter fungi grew and sporulated as the controls after 14 days of exposure to the two herbicides at the 1X rate and 25°C.

Development (mycelial growth and sporulation intensity) of the four Hyphomycetes was usually inhibited to various degrees on agar treated with 0.1X of either phenmedipham plus desmedipham or metolachlor; sporulation intensity of *P. farinosus* was enhanced at 25°C (*Table 3*). The antibiotic/fungistatic effects of the herbicides on the Hyphomycetes were suppressed by subculturing on herbicide-free agar.

### Chloridazon

Although *C. thromboides* was killed at 0.1X of chloridazon (a pyridazinone), only strong (*C. coronatus* at 15°C) to usually moderate inhibition of growth occurred with the other fungi at the same rate (*Table 3*). Sporulation intensity levels varied from much lower (100% reduction for *M. anisopliae* at 15°C) to much higher (200% enhancement for *P. farinosus* at 25°C) compared with controls. The inhibitory effect on development of the five fungi was not expressed in subcultures on untreated agar.

At both temperatures, chloridazon was fungicidal to all six fungi at the 1X and 10X rates (*Tables 2* and *4*). In each case, the fungicidal effect was maintained on inocula transferred to untreated agar.

### Lenacil

Lenacil (a pyrimidine) was usually the least antagonistic of the herbicides tested against the fungi, particularly at 25°C. At the 1X and 10X rates, however, it was fungicidal to *C. thromboides* at 15°C (*Tables 2* and *4*). The effect was not overcome on untreated agar. At the 0.1X rate the herbicide completely inhibited the development of *C. thromboides* at 15°C until between days 7 and 9 (data not shown); thereafter, the fungus gradually surmounted the inhibition (*Table 3*) and developed as in the controls. At 25°C, strong, although reversible (on untreated agar), inhibition of *C.*

Table 2. Radial growth (G)[a] and sporulation intensity (S)[b] of entomopathogenic fungi recorded after 14 days (at 25°C) and 24 days (at 15°C) of incubation on Sabouraud dextrose agar treated with the recommented rate (1X) of herbicides

| | Herbicide and temperature (°C) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Phenmedipham + desmedipham 2% | | | | Metolachlor 0.5% | | | | Chloridazon 1% | | | | Lenacil 0.25% | | | |
| | 15 | | 25 | | 15 | | 25 | | 15 | | 25 | | 15 | | 25 | |
| | G | S | G | S | G | S | G | S | G | S | G | S | G | S | G | S |
| *C. coronatus* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 45.5 (0.8) | 89.2 | 100 (0.0) | 4.0 |
| *C. thromboides* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 100 (0.0) | 67.9 |
| *B. bassiana* | 0 | 100 | 0 | 100 | 0 | 100 | 11.9 (0.5) | 100 | 0 | 100 | 0 | 100 | 82.6 (0.8) | 46.4 | 69.2 (0.8) | [3.8] |
| *M. anisopliae* | 0 | 100 | 0 | 100 | 0 | 100 | 12.4 (0.5) | 76.6 | 0 | 100 | 0 | 100 | 98.4 (0.8) | 96.0 | 74.8 (0.5) | 31.6 |
| *P. farinosus* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 71.4 (0.8) | 56.4 | 94.7 (0.9) | 1.4 |
| *V. lecanii* | 0 | 100 | 0 | 100 | 0 | 100 | 16.7 (0.8) | 95.5 | 0 | 100 | 0 | 100 | 93.6 (0.9) | 56.2 | 82.8 (0.8) | 93.3 |

[a]Radial growth expressed as percentage of growth on acetone-treated agar (control = 100%); mean [± standard error (in parentheses)] of four replicates; [b]expressed as percentage of reduction [enhancement in squared brackets] in production of viable spores $cm^{-2}$ of fungal colony compared with control

Table 3. Radial growth (G)[a] and sporulation intensity (S)[b] of entomopathogenic fungi recorded after 14 days (at 25°C) and 24 days (at 15°C) of incubation on Sabouraud dextrose agar treated with 0.1X rate of herbicides

| | Herbicide and temperature (°C) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Phenmedipham + desmedipham 2% | | | | Metolachlor 0.5% | | | | Chloridazon 1% | | | | Lenacil 0.25% | | | |
| | 15 | | 25 | | 15 | | 25 | | 15 | | 25 | | 15 | | 25 | |
| | G | S | G | S | G | S | G | S | G | S | G | S | G | S | G | S |
| *C. coronatus* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 10.5 (0.6) | 100 | 88.6 (1.3) | 100 | 100 (0.0) | 78.7 | 100 (0.0) | [21.1] |
| *C. thromboides* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 53.7 (1.4) | 66.0 | 100 (0.0) | [3.5] |
| *B. bassiana* | 19.6 (0.6) | 100 | 21.7 (0.6) | 92.8 | 51.7 (0.5) | 63.2 | 63.9 (0.5) | 52.6 | 50.4 (1.2) | [3.6] | 56.7 (1.2) | 42.7 | 69.3 (0.5) | 36.0 | 90.7 (0.9) | [64.8] |
| *M. anisopliae* | 0 | 100 | 22.0 (0.6) | 100 | 49.0 (0.6) | 100 | 26.7 (0.8) | 53.0 | 44.2 (0.6) | 100 | 55.2 (1.3) | 55.0 | 111.5 (0.8) | 90.9 | 97.3 (1.2) | [8.3] |
| *P. farinosus* | 37.9 (0.6) | 49.3 | 36.6 (0.6) | 54.2 | 51.6 (0.6) | 23.4 | 59.8 (2.0) | [47.0] | 61.0 (0.5) | 28.2 | 70.3 (1.6) | [200.3] | 92.1 (0.9) | 16.1 | 100 (0.8) | [90.2] |
| *V. lecanii* | 38.0 (0.6) | 21.3 | 36.3 (0.5) | 92.6 | 50.9 (0.5) | 55.9 | 53.9 (0.8) | 93.0 | 64.9 (1.4) | 21.3 | 66.0 (2.9) | 88.7 | 92.0 (1.2) | 30.1 | 94.9 (0.8) | 89.2 |

[a,b]As in *Table 2*

Table 4. Radial growth (G)[a] and sporulation intensity (S)[b] of entomopathogenic fungi recorded after 14 days (at 25°C) and 24 days (at 15°C) of incubation on Sabouraud dextrose agar treated with 10X rate of herbicides

| | Herbicide and temperature (°C) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Phenmedipham + desmedipham 2% | | | | Metolachlor 0.5% | | | | Chloridazon 1% | | | | Lenacil 0.25% | | | |
| | 15 | | 25 | | 15 | | 25 | | 15 | | 25 | | 15 | | 25 | |
| | G | S | G | S | G | S | G | S | G | S | G | S | G | S | G | S |
| *C. coronatus* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 9.2 (0.5) | 100 | 30.0 (1.4) | 100 |
| *C. thromboides* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 10.0 (0.6) | 100 |
| *B. bassiana* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 45.7 (0.8) | 79.3 | 52.7 (0.7) | 30.7 |
| *M. anisopliae* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 67.2 (0.5) | 100 | 82.3 (0.8) | 90.1 |
| *P. farinosus* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 50.0 (0.6) | 84.8 | 94.7 (0.9) | 72.7 |
| *V. lecanii* | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 61.6 (0.9) | 100 | 69.2 (0.9) | 96.1 |

[a,b]As in *Table 2*

*thromboides* was observed only at the 10X rate of lenacil (*Table 4*). Radial growth at the 1X and 0.1X rates was equal to the controls (*Tables 2* and *3*). Sporulation intensity was, however, markedly curtailed at the 1X rate (*Table 2*).

From 45.5% (at 15°C) to no inhibition (at 25°C) of growth of *C. coronatus*, and 100% reduction (at 15°C) in sporulation intensity occurred at the 1X rate of lenacil (*Table 2*). *Conidiobolus coronatus* grew normally at both temperatures on agar treated with 0.1X of the herbicide; sporulation intensity was lessened by 78.7% at 15°C and enhanced by 21.1% at 25°C (*Table 3*). Initially, complete (data not shown) and then pronounced fungistasis, and marked reduction in sporulation intensity, resulted from exposure to the 10X rate of herbicide at both temperatures (*Table 4*). The fungus exposed to the three rates of lenacil developed typically, once subcultured on untreated agar.

Lenacil had no fungicidal activity against any of the four Hyphomycetes. The effects of the herbicide on fungus vegetative growth ranged from complete, although temporary, inhibition to gradually increasing or decreasing inhibition, to slight stimulation (*M. anisopliae* at the 0.1X rate and 15°C). A general growth pattern for the four fungi was not found; each fungus responded in a slightly different way to different lenacil concentration–temperature combinations (*Tables 2–4*). Retardation or stimulation of growth of the four fungi was not observed in subcultures on lenacil-free agar. Sporulation intensity on agar treated with the 1X and 0.1X rates of lenacil was usually reduced, but was enhanced in a few cases at 25°C (*Tables 2* and *3*).

## Discussion

Phenmedipham plus desmedipham and metolachlor were found to be 'harmful' to the Hyphomycetes

tested, with the exception of metolachlor which was 'slightly harmful' to *M. anisopliae* and 'moderately harmful' to *V. lecanii* (*Table 5*), according to Hassan's scheme (Hassan, 1989), and to Hokkanen and Kotiluoto's bioassay procedure (Hokkanen and Kotiluoto, 1992), which requires testing of pesticides at the highest concentration recommended for field use (1X) and at 22–24°C. Both herbicides were classified as 'harmful' to *C. coronatus* and *C. thromboides* (*Table 5*). Chloradizon at the recommended field rate was classified as 'harmful' *in vitro* to the six fungi tested (*Table 5*). Lenacil was classified as 'slightly harmful' to *C. thromboides*, 'moderately harmful' to *V. lecanii*, and 'harmless' to the other fungi tested (*Table 5*).

Whereas products found to be harmful *in vitro* will not necessarily cause damage to field populations of entomopathogenic fungi, harmless chemicals are very likely to remain so in the field. Nevertheless, because the reduction in beneficial capacity (intensity of production of viable spores, e.g. of infective inoculum) in all six fungi was >50% (*Table 5*), phenmedipham plus desmedipham, metolachlor and chloradizon need to be tested further under semi-field conditions before a conclusion on their real effect on the fungi can be drawn (Hassan, 1989). The apparent moderate to high level of tolerance to lenacil of *C. coronatus*, *B. bassiana*, *M. anisopliae* and *P. farinosus* (the latter three species operating mainly in the soil environment), indicate that these fungi and this herbicide may be utilized if applications are scheduled to minimize negative interactions. Further testing including bioassays *in vivo* is needed with *C. thromboides* and *V. lecanii*, which are species infecting pest populations mainly in the field canopy.

Three points, in particular, emerged from our study. First, there was rarely stimulation of fungal growth and enhancement of quantity of viable spores produced. Herbicide interference in fungal growth included suppression (or delaying) or spore germination, inhibition of the linear extension of the mycelium, and abnormalities in growth habit and in patterns of spore production. Second, three herbicides (phenmedipham plus desmedipham, metolachlor and chloridazon), were more fungitoxic than lenacil to a range of organisms. The inhibitory and fungicidal effects were observed at rates of active ingredients well within the range likely to be expected in the field. Third, the susceptibility of entomopathogenic fungi to herbicides varied between chemicals and between fungal species. Generally, adverse effects of the herbicides were greater against the Entomophthorales than against the Hyphomycetes.

We do not have, and have not found published, supporting data to suggest possible reasons for the variation in response of the different test fungi to the different herbicides. The differential responses by Hyphomycetes and Entomophthorales to herbicides appear to underscore significant phylogenetic differences between the two groups of fungi. Similar differential responses by these groups of entomopathogenic fungi to fungicides have been reported (Majchrowicz and Poprawski, 1993).

We have not investigated the complex research area on the mode of action of herbicides on the fungi. The modes of action of herbicides on higher plants have been reviewed (Casely, Atkin and Cussans, 1991), but any extrapolation to entomopathogenic fungi would be conjectural. Although the mode and site of action have not been established, inhibition *in vitro* of phytopathogenic fungi with herbicides has been reported for several pathogens and herbicides (Casely *et al.*, 1991). Reviews on the effect of herbicides on plant disease indicate that the incidence of mycoses may be either increased or decreased by the herbicides. In general, the effects of herbicides in altering plant mycoses include alteration of the host-plant defences, stimulation of increased exudation from host plants and alteration of pathogen growth.

There are several extensively reviewed and controversial issues relating to the degree of resistance/tolerance to chemical pesticides in arthropod predators and parasitoids (Theiling and Croft, 1988; Hoy, 1990, and references cited). There is less information with regard to entomopathogenic fungi, and most of it deals with fungicides and insecticides. Almost nothing is known about the effect of herbicides on these fungi (Keller, 1986). Although, in our tests, several fungi were able to overcome to some degree the herbicide inhibitory effect, it is improbable that pesticide resistance was involved because pesticide resistance implies a genetically induced change in the ability of a population to tolerate pesticides. The term 'tolerance' is more appropriate because it describes the ability of an organism to survive a specific pesticide dose; it does not imply that a genetically determined change has

Table 5. Evaluation categories [a] of the safety of entomopathogenic fungi of four herbicides tested *in vitro* at the recommended rate (1X) of active ingredient, at two different temperatures

| | Herbicide and temperature (°C) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Phenmedipham + desmedipham 2% | | Metolachlor 0.5% | | Chloridazon 1% | | Lenacil 0.25% | |
| Fungus | 15 | 25 | 15 | 25 | 15 | 25 | 15 | 25 |
| *C. coronatus* | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 1 |
| *C. thromboides* | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2 |
| *B. bassiana* | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 1 |
| *M. anisopliae* | 4 | 4 | 4 | 2 | 4 | 4 | 3 | 1 |
| *P. farinosus* | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 1 |
| *V. lecanii* | 4 | 4 | 4 | 3 | 4 | 4 | 2 | 3 |

[a]Evaluation categories: 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50–79%), 3 = moderately harmful (80–99%), 4 = harmful (>99%) (Hassan, 1989); evaluation based on the percentage of reduction in number of viable spores produced compared with control, 14 days (at 25°C) and 24 days (at 15°C) after treatment (see *Table 2*) (Hokkanen and Kotiluoto, 1992)

occurred. The mechanisms involved in fungistasis can not be deduced from the data collected in our study. However, the delayed mycelial growth response, obviously resulting from delayed spore germination, observed in some combinations, supports the suggestion that there is an induced detoxification system to deal with herbicides in these fungi and thus that the effect of the herbicides on the fungi is restricted to spore germination. It is also possible that the processes of spore germination and vegetative hyphal growth of a given fungus have differing sensitivities to a given herbicide. Taking metolachlor as an example, a possible interpretation is that it killed a large number of spores (see Keller, 1986). Some spores (*B. bassiana*, *M. anisopliae* and *V. lecanii*, at the recommended rate) survived, but obviously a small number of germinated spores would require much more time to produce a measurable colony than the control. On the other hand, there was indication of a constant growth rate on herbicide-treated agar, although this was substantially lower than the control. The breakdown of active ingredients with time might explain delayed mycelial growth. Clearly, further research is needed on the mechanisms involved in fungal resistance/tolerance to herbicides and other pesticides. Roberts and Campbell (1977) suggested that strain selection for pesticide tolerance and compatibility may improve biological control potential.

The effects of herbicides on entomopathogenic fungi *in vitro* do not necessarily reflect field effects. The tests reported here have been performed on artificial media and may not give more than tentative answers concerning the influence of pesticides in living arthropod–fungal pathogen systems. It appears however that several herbicides have a detrimental effect on entomopathogenic fungi (Ignoffo, *et al.*, 1975; Gardner and Storey, 1985; Keller, 1986; Vänninen and Hokkanen, 1988; Mietkiewski, Sapiecha and Mietkiewska, 1989; Glare and Milner, 1991; Keller and Schweizer, 1991; Harrison and Gardner, 1992) and their application would reduce these fungal populations in a crop before they infect arthropods (Ignoffo *et al.*, 1976). Entomopathogenic fungi are generally very sensitive to fungicides and also to some herbicides and it is likely that their role in the natural mechanisms of insect regulation is, or may be, seriously hindered by the increasing use of such pesticides (Keller, 1986, 1991).

We believe that only lenacil, of the four herbicides tested by us, could be used selectively in integrated control programmes where fungal entomopathogens are to be used. However, our results are based on effects of various concentrations of the chemical on agar plates, and consequently extrapolation to the field is very difficult. Agar media may shadow any toxic effect of the chemical. Our data also suggest a potential incompatibility of chloridazon, metolachlor and phenmedipham plus desmedipham with insect pest management practices. The toxicities of the latter to entomopathogenic fungi should be further assessed *in vivo* in the laboratory and under field conditions.

Pesticide effects can be studied under simulated conditions in the laboratory. However, care needs to be taken in extrapolating into field situations unless the doses are very precisely known and all aspects of the cycle have been studied. Hassan *et al.* (1991) stated that pesticides found to be harmless to a particular organism in initial toxicity testing in the laboratory need no further testing in semi-field or field experiments on the routine basis. Considering the complexity of factors involved, it is evident that reliance on only those measures of pesticide effects most commonly used *in vitro* will continue to generate data of limited value. However, although initial laboratory studies cannot assess the probability in the field of fungus–herbicide interaction and resultant fungus risk, laboratory findings can determine additional research needed. The relationship between laboratory toxicity and field performance of pesticides has been described as 'generally unexplored' (Hoy, 1990), yet this relationship appears to be critical in determining whether pesticides are selective to natural enemies. We have much to learn about how to standardize, assay, interpret data and select for pesticide resistance/tolerance in arthropod natural enemies, including fungal pathogens. Consideration of the conservation of natural enemies will often conflict with necessary control measures of pest populations. This situation is frequently encountered when early season applications of pesticides endanger the biocontrol element, and commit the crop to chemical protection for the remainder of the season (Havron *et al.*, 1987). It is difficult to generalize about compatibility among entomopathogenic fungi and pesticides for pest control. Entomophthoralean entomopathogenic fungi, being obligate parasites, are probably more susceptible to herbicides than are entomopathogenic Hyphomycetes, which are facultative parasites. At present, there appears to be a compromise between using pesticides and the detrimental effect that this can have on natural enemies (Haynes, Tummala and Ellis, 1980).

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This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation of this product by the US Department of Agriculture. We thank with appreciation R. I. Carruthers, R. A. Humber, S. B. Krasnoff and B. C. Legaspi for their valuable suggestions in reviewing the manuscript.

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